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Analysis of phaseolin and total storage protein-based diversity in common bean landraces of Nilgiri using SDS-PAGE

Franklin Charles Jose*, M. M. Sudheer Mohammed[1], R. Sanil[2], M. Dorai and M. G. Madanan[3]

Department of Plant Biology and Biotechnology, [2]Department of Advanced Zoology and Wildlife Biology, Government Arts College, Stone House Hill P.O., Udhgamandalam, Nilgiris 643 002
[1]Department of Plant biology and Biotechnology, Government Arts College (Autonomous), Coimbatore 641 018
[3]Regional Medical Research Center (ICMR), P.B. No. 13, Dollygunj, Port Blair 744 101, Andaman & Nicobar Islands

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Abstract

Seed storage protein variation was studied in 20 common bean landraces (LRs) collected from different traditional farming villages of Nilgiris district of Tamilnadu, India, with Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Evaluation of common bean germplasm with seed storage proteins is essential for conservation, breeding and to determine the possible origin of common beans. The electrophoretogram revealed major seed proteins such as phaseolin (PHS), Phytohemagglutinin (PHA), α-amylase inhibitors (αAIs) and Arcelin (ARL). The purified PHS fraction has three and two subunits, respectively in the Andean and Mesoamerican landraces. A dendrogram was constructed based on Jaccard's coefficients of protein markers using the average distance method, which has separated the accessions into two major clusters, eight Mesoamerican and twelve Andean. This result proved that Nilgiris' common bean population is highly diverse and there is immense scope for further improvement. A better knowledge of seed storage protein variation of common beans will help in genetic improvement and conservation programme for its landraces in Nilgiris.

Key words: Biodiversity, Germplasm conservation , Nilgiris, *Phaseolus vulgaris*, Phaseolin, Seed storage protein

Introduction

The common bean (*Phaseolus vulgaris* L.) was domesticated in two distinct regions of the New World, one in Mesoamerica and another along the Eastern slope of the Andes in South America nearly 8000 years ago [1, 2]. Common bean is the most important cultivated species of the genus *Phaseolus* and is a major source of dietary protein for millions of people across the globe [2]. The crop is consumed principally for its dry (mature) beans and green pods. In India, beans are grown in an area of about nine million hectares with an annual production of three million tonnes [3]. Common beans grown all over Himachal Pradesh possess extreme morphological variability. The Himalayas represent the richest repository of bean germplasm in India. Accessions of common beans from Srinagar, Jammu, Kishtwar and Kulu areas of Northern India were evaluated with regard to phaseolin protein [4]. However, no literature is available regarding the common bean landraces from Western Ghats in general and Nilgiris biosphere reserve in particular. Hence, the present study aimed to investigate seed storage protein variation in general and phaseolin in particular among 20 landraces of common beans collected from the traditional tribal farming villages of Nilgiris.

Materials and methods

Plant material

The landraces of common bean were raised from seeds collected from traditional farming villages of Nilgiris. The twenty landraces have been identified based on seed size, coat colour, size, shape and colour pattern [5] (Table 1). The collected plants were evaluated as per International Board of Plant Genetic Resources descriptors for *Phaseolus vulgaris* L. [6].

Total protein SDS-PAGE

Seed coats and embryos were manually removed from dry seeds. The cotyledons, which contain the storage proteins, were dried in an oven at 40°C. 100 mg of seed flour was weighed out and proteins were extracted with 10mL of 0.05M Tris-HCl buffer, pH 6.7 and centrifuged

*Corresponding author's e-mail: fcjooty@yahoo.com

Table 1. *Phaseolus vulgaris* L. landraces from Nilgiris used in this study

Accession No.	Local name/Market class	Village/place of collection	Altitude m (amsl)	Growth habit (I*,III**,IV***)
LR1	Cranberry bean	Nanjanadu	2376	I
LR2	Black turtle	Ooty	2376	I
LR3	Hatti avarai	Adigaratti	2261	I
LR4	Vartex	Tumanatti	1920	I
LR5	White kidney	Nanjanadu	2376	I
LR6	Red kidney bean	Ozahatti	2261	I
LR7	Black bean	Nanjanadu	2376	III
LR8	Curry bean	Ebanad	1930	III
LR9	White eyed bean	Ooty	2376	III
LR10	Hatti avarai	Nundala	2180	IV
LR11	Earpai avarai	Nanjanadu	2376	IV
LR12	Hatti avarai	Tuneri	2066	IV
LR13	Pinto bean	Muttinadu	2200	IV
LR14	Thol avarai	Sholur	2277	IV
LR15	Kuruvi avarai	Wellington	2041	IV
LR16	Butter bean	Bikkatti	2532	IV
LR17	Bush bean	Nanjanadu	2376	I
LR18	Kaki avarai	Kokkal	2270	IV
LR19	Curry bean	Ebanad	2060	IV
LR20	Mysore avarai	Muttinadu	2200	III

*-Type I growth habit (Dwarf plants); **-Type III growth habit (tall-weak climbers); ***-Type IV growth habit (tall-strong climbers)

at 10,000 rpm for 10min. The supernatant was added with five volumes of chilled acetone and kept at -20° C for 10 hrs. Acetone was removed by centrifugation at 4°C and the protein pellet was dissolved in electrophoretic sample buffer (0.05M Tris pH 6.8, SDS 10%, α-mercaptoethanol, glycerol 10% and water 20%) and kept at room temperature. SDS-PAGE was carried out according to the method of Laemmli [7] in a vertical slab gel electrophoretic apparatus (Bangalore Genei).

Phaseolin SDS-PAGE

Phaseolin was purified from 200mg of seed flour, placed in a 1.5ml plastic tube, and 1mL of extracting solution (0.5M NaCl, 0.25M ascorbic acid, pH 2.4) was added. The mix was kept in the dark with agitation for 1 hour. The samples were then centrifuged at 10,000 rpm for 20 minutes. In order to achieve maximum extraction of phaseolins from the samples, the previous steps were repeated twice. After each centrifugation, the supernatant was decanted in to a 10mL test tube. After performing the third extraction, added five volumes of water at 4°C to the test tubes. As phaseolins are not soluble in weak saline solution, they precipitated with the addition of water. To achieve maximum precipitation of phaseolins, the tubes were kept in the dark at 4°C for 30 minutes and then centrifuged for 20 minutes. The precipitate was then resuspended in a known volume of electrophoretic sample buffer and SDS-PAGE was carried out.

Data analysis

The apparent molecular weights determination was accomplished through the medium range SDS-PAGE markers (14.3 to 97.4 kD; Genei, Bangalore). The evaluation was made by observing the presence (1) or absence (0) of bands of the same molecular weight, and a binary matrix was constructed. These values were used to assess genetic relationships based on Jaccard's similarity index. Jaccard's coefficients were used to construct a dendrogram using UPGMA using the

software MVSP version 3.13n (Multivariate Statistical Package; http://www.kovcomp.com/mvsp).

Results and discussion

The gel was evaluated visually and the protein bands were identified in comparison with standard molecular weight markers (Lysozyme-14.3 kD, Soya bean trypsin inhibitor-20.1 kD, Carbonic anhydrase-29 kD, Ovalbumin-43 kD, Bovine serum albumin-66 kD and Phosphorylase b-97.4 kD). In total seed protein gel (Figure 1) there were six types of major banding patters. Intensive bands within the range of 54 kD to 43 kD represent the major seed storage protein, phaseolin. Phaseolin patterns are used to identify gene pools and the origin of varieties referring to the Mesoamerican or Andean domestication centers [1]. Populations from Middle America usually have small seeds and S-type (Sanilac) phaseolin (Fig. 2B) with two prominent bands in the phaseolin [8]. The South American ones generally show medium sized and large seeds with T-type (Tender green) with three bands [1] in the phaseolin (Fig. 2A).

Out of 20 landraces evaluated for phaseolin variation, 12 landraces (LR1, LR3, LR4, LR6, LR10, LR11, LR12, LR13, LR15, LR16, LR17 and LR18) are with T- type phaseolin and eight landraces (LR2, LR5, LR7, LR8, LR9, LR14, LR19 and LR20) are with S-type phaseolin. Baring 'T' and 'S' type phaseolins, no other variant could be detected in this collection. The prevalence of high frequency of Andean races with T - phaseolin was reported by Zeven *et al.* [9]. High proportion of landraces with 'T' phaseolin is also reported in many parts of Europe, a secondary center of common bean domestication [10, 11]. Moreover, types belonging to the Andean gene pool, possessing medium and large seeds, are more largely widespread since they are preferred by both farmers and consumers [12, 13]. The Andean genotypes may be better adapted to the cool climatic conditions of Nilgiris.

The remaining low molecular weight proteins are albumins; which include arcelin (ARL), phytohemagglutinin (PHA), and α-amylase inhibitors (α-AIs) in the descending order of molecular weight (Fig. 1).

Arcelin protein is the characteristic feature of Mesoamerican genepool. There are exceptions, however, which is the result of introgression. The Mesoamerican germplasm as a source of resistance to bruchids, is llustrated by the arcelin protein. This resistance was absent in thousands of populations of domesticated common bean, while only a few wild bean populations from Mexico were highly resistant [14]. ARL is in the 35kD region, which is present in eight Mesoamerican landraces (LR2, LR5, LR7, LR9, LR11, LR14, LR19 and LR20). But contrary to the general hypothesis, the Mesoamerican variety LR8 did not have this protein, but the Andean LR11 did possess this protein. This reveals the introgression of seed protein genes among the common bean landraces in Nilgiris. These hybrid plants can be used as parents to introgress genes from different genepools in future bean improvement programmes.

The PHA fraction is in the 34 kD–30 kD range, represented by a single band, in all the landraces, but with different intensities (Fig. 1). PHAs are seed lectins that bind carbohydrates in a reversible and specific manner, and often have hemagglutinating properties [15]. Although the function of PHA has not been demonstrated conclusively, but it suggests that a major part of its adaptive significance is to protect bean seeds from insect predators. Therefore, this protein could be an important candidate molecule for further evolution

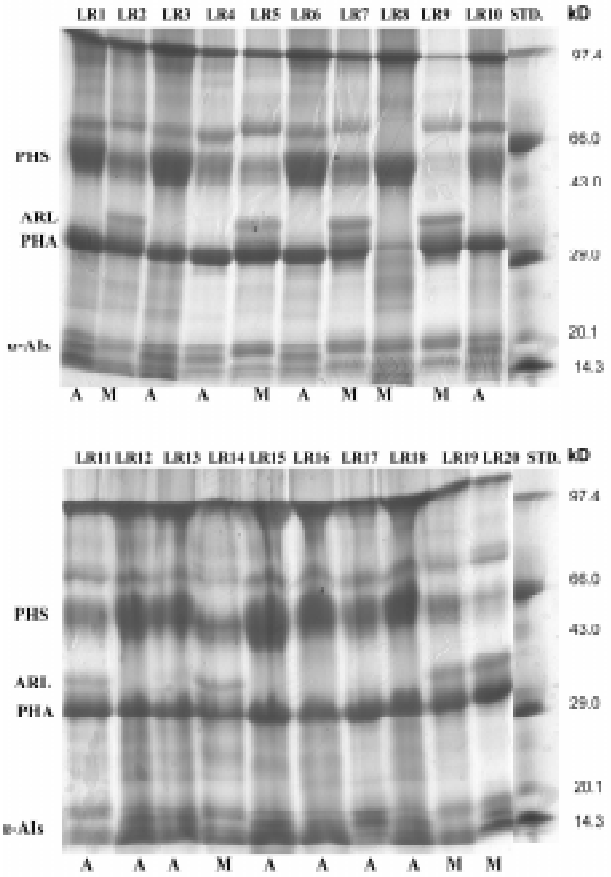

Fig. 1. SDS-PAGE of total seed storage proteins: A-Andean, M-Mesoamerican

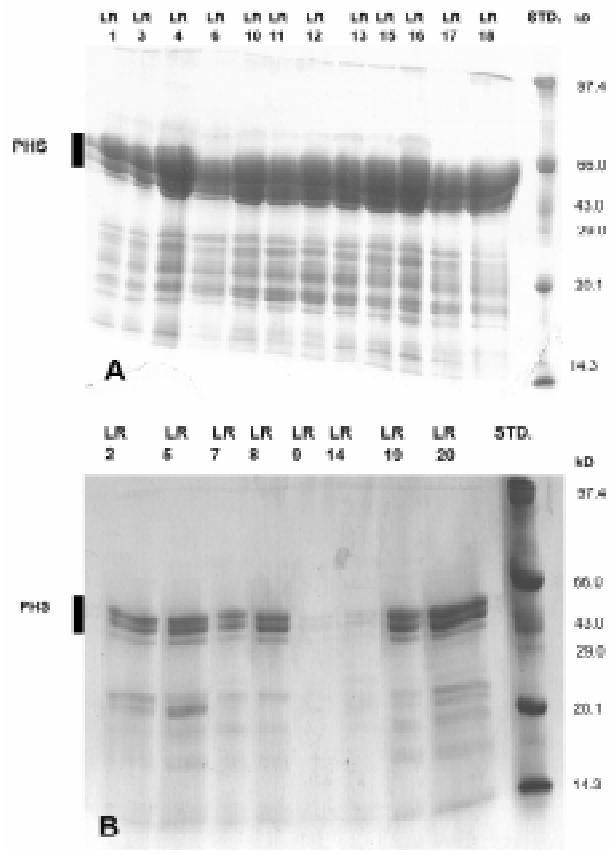

Fig. 2. A. SDS-PAGE of phaseolin in Andean races B. -SDS-PAGE of phaseolin in Mesoamerican races

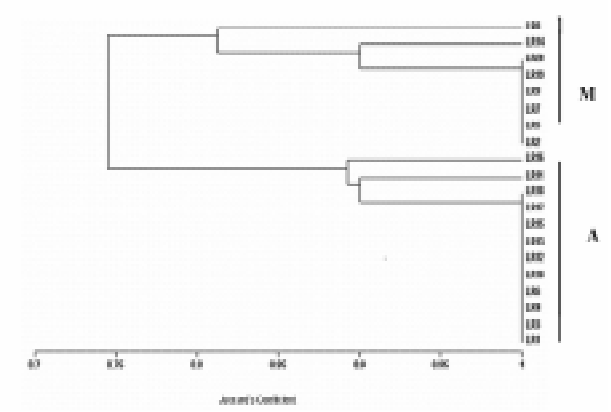

Fig. 3. Dendrogram based on Jaccard's similarity coefficients of SDS-PAGE data. M-Mesoamerican races genepool; A-Andean genepool

to new environment. α-AIs are 18 kD-14 kD proteins which showed difference in landraces studied (Fig. 1). The αAIs inhibit the enzymes present in the intestinal tracts of some insects such as bruchids and other storage product pests [16].

The dendrogram (Fig. 3) constructed from Jaccard's coefficients clearly separated the landraces into two distinct clusters- Mesoamerican and Andean with three subclusters each. The 'M' cluster includes eight landraces (LR2, LR5, LR7, LR8, LR9, LR14, LR19 and LR20). The 'A' cluster consists of twelve landraces (LR1, LR3, LR4, LR6, LR10, LR11, LR12, LR13, LR15, LR16, LR17 and LR18). The 'M' cluster was found to be more diverse than the 'A' cluster.

These results thus indicated existance of seed storage protein variation within the landraces of common beans in Nilgiris. The level of polymorphism observed in the present study was moderately high, indicating a wide and diverse genetic base for the common bean landraces which maintain a higher intraspecific genetic diversity in Nilgiris. There exists a clear-cut sign of introgression between the two gene pools as indicated by the hybrids, merit further evolutionary investigation.

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